Influence of Complex Formation on Membrane Transport

V. S. VAIDHYANATHAN

Abstract □ Theoretical aspects of the influence of complex formation on transport across a diffusional barrier of three species, participating in an association–dissociation reaction, are presented. On the basis of solution of a nonlinear differential equation derived from equations of continuity, valid for stationary states, when diffusion coefficients are constants and coupling between fluxes of different species is ignored, it is shown that the usual assumption that the complex formation reaction is at equilibrium at all locations in the system is not valid, except when the concentration of one of the reactant species is maintained equal on both sides of the membrane. In the general case, methods of calculation of reaction-rate profiles and concentration profiles to any desired order of approximation in the inhomogeneous diffusional barrier region from experimentally measurable quantities are included. The method of computing the association-rate and dissociation-rate constants from suitable transport measurements is presented.

Keyphrases □ Kinetics—complex formation membrane transport, theoretical □ Membrane transport—complex formation effect, theoretical □ Complex formation—effect on membrane transport, kinetic theory □ Association-, dissociation-rate constants—computation methods □ Reaction, concentration-rate profiles, inhomogeneous barrier region—calculation

In a series of papers on the effect of complex formation on drug absorption, Reuning and Levy (1–3) presented experimental information and analysis of a model describing the overall transfer of a drug across a diffusional barrier in the presence of a complexing agent. Their experimental setup is that specified concentrations of a mixture of α (caffeine) and β (salicylamide) are placed in a conical flask of relatively large volume and well stirred. A small amount of solution containing β is placed in a nylon bag and suspended in the large volume mixture. Samples of solution in the nylon bag are withdrawn at regular time intervals and analyzed for total concentrations of α and β in the nylon bag.

The basic assumption in these experiments is that α and β, when present together in solution, form a complex γ represented by the reaction:

$$\alpha + \beta \underset{k_2}{\overset{k_1}{\rightleftharpoons}} \gamma \qquad \text{(Eq. 1)}$$

where k_1 and k_2 are the rate constants for the association–dissociation reaction. The equilibrium constant, $K = (k_1/k_2)$, is determined by various methods to be 30–50 l./mole. It is assumed that the reaction represented by Eq. 1 is at equilibrium at all locations in the system and that the species transport across the diffusional barrier at a rate proportional to their respective concentration gradients. Experimentally, it is determined that species α permeates the diffusional barrier much more slowly than species β.

A series of experiments with varying initial concentrations of β in the nylon bag and specified concentration of α and β in the outside solution was performed; the time variation of concentrations of β in the nylon bag was determined. The fraction of total amount of β present in complexed form was computed and utilized in the calculation of the stability constant for complex formation.

The principal objectives of this paper are to: (*a*) analyze the significance of experimental information obtained by the above-mentioned set of experiments, (*b*) examine the validity of the basic assumptions invoked in the interpretation of experimental data, and (*c*) present certain theoretical aspects of the influence of chemical reactions on the flux of species across a diffusional barrier which also participate in a reaction of the type presented in Eq. 1. If the reaction presented in Eq. 1 is not at equilibrium, then, by necessity, the reaction-rate profile $J_R(x)$.

$$J_R(x) = k_1 C_\alpha(x) C_\beta(x) - k_2 C_\gamma(x) \qquad \text{(Eq. 2)}$$

will be a function of position variable x in the inhomogeneous diffusional barrier under conditions of stationary states and nonsteady states. In Eq. 2, $C_\sigma(x)$ is the local concentration of species σ at a specified location x in the system. The position variable x is defined along a direction normal to the plane of the diffusional barrier. Both $J_R(x)$ and $C_\sigma(x)$ are independent of time under conditions of steady state and vary with time under nonsteady-state conditions.

Later in this paper, expressions for the reaction-rate profile for nonsteady states and steady states are presented. In the section, *Nonstationary-State Conditions*, the general aspects of the problem as dictated by equations of continuity are discussed. In the section, *Stationary State (Reaction at Equilibrium)*, considerations valid for steady states when the reaction is at equilibrium at all locations of the diffusional barrier are presented. In that section, it is shown that unless the concentration of one of the species, α or β, is maintained the same in solutions on both sides of the diffusional barrier, the reaction-rate profile will be nonvanishing in the inhomogeneous region. The inhomogeneous character of the diffusional barrier is responsible for the maintenance of a stationary-state reaction-rate profile. In a later section, an expression is presented for the reaction-rate profile valid in the inhomogeneous region when diffusion coefficients are regarded as constants and when coupling between fluxes of different species may be ignored. One may compute the reaction-rate profile and the effect of chemical reaction on flux of a specified species across a diffusional barrier.

Reuning and Levy (1–3) considered the possible occurrence of dimerization of α and the possibility of a 2:1 complex formation, in addition to the reaction of the type presented in Eq. 1, occurring in the system. For the sake of brevity, it is assumed that only the reaction represented by Eq. 1 occurs in the system presented here.

NONSTATIONARY-STATE CONDITIONS

It is both interesting and illuminating to begin with the conditions valid for nonstationary state. Experiments of the type mentioned previously yield measurement of (dC_σ^{II}/dt), where C_σ^{II} is the concentration of species σ in the nylon bag and t is the time variable. In the following, time derivatives are denoted with a super dot and quantities corresponding to stationary states are denoted with an asterisk to distinguish them from corresponding quantities of nonsteady-state conditions. When reaction of the type represented in Eq. 1 takes place in the system, one has the equations of continuity for the three species:

$$\dot{C}_\alpha = -\nabla\cdot J_\alpha(x) - J_R(x) \qquad \text{(Eq. 3}a\text{)}$$

$$\dot{C}_\beta = -\nabla\cdot J_\beta(x) - J_R \qquad \text{(Eq. 3}b\text{)}$$

$$\dot{C}_\gamma = -\nabla\cdot J_\gamma + J_R \qquad \text{(Eq. 3}c\text{)}$$

where J_σ is the matter flux (in moles per unit area per unit time) of species σ. So long as one considers only one-dimensional transport, *i.e.*, transport along an axis normal to the plane of the membrane, one may replace $\nabla\cdot J_\sigma$ by $(dJ_\sigma/dx) = J_\sigma'$. The first derivative with respect to position variable is denoted by a single prime. The second derivative with respect to x is denoted by a double prime.

Within the spirit of irreversible thermodynamics and existence of nonvanishing interactions between molecules across space, the flux of a molecule of a specified species is correlated with the position and fluxes of all other molecules present in the system (4–9). If one now assumes that the influence of fluxes of other species on the flux of a specified species may be ignored and that the flux of a specified species is proportional to its concentration gradient as a first approximation, in the spirit of Fick's equation, one may write:

$$J_\sigma = -D_\sigma C_\sigma' \qquad \text{(Eq. 4)}$$

where D_σ is the diffusion coefficient of species σ in the system. In principle, D_σ is dependent on concentration profiles of all species present in the inhomogeneous system, given by complicated expressions of molecular theory (10). Thus, the diffusion coefficient is a function of the position variable. If one adopts, as a second approximation, the assumption that diffusion coefficients may be regarded as constants, one may rewrite the equations of continuity as:

$$\dot{C}_\alpha = -D_\alpha C_\alpha'' - J_R \qquad \text{(Eq. 5}a\text{)}$$

$$\dot{C}_\beta = -D_\beta C_\beta'' - J_R \qquad \text{(Eq. 5}b\text{)}$$

$$\dot{C}_\gamma = -D_\gamma C_\gamma'' + J_R \qquad \text{(Eq. 5}c\text{)}$$

Under steady-state conditions, all explicit time derivatives must vanish. Thus one recovers a basic equation valid for stationary states:

$$-J_\alpha' = -J_\beta' = J_\gamma' = J_R \qquad \text{(Eq. 6)}$$

Equation 6, along with the assumed validity of Eq. 4, formed the basis for the derivation of a (nonlinear) differential equation for the reaction-rate profile by Blumenthal and Katchalsky (11)—*viz.*,

$$J_R^*(x) = \lambda^{-2}J_R^* + 2k_1C_\alpha'C_\beta' \qquad \text{(Eq. 7)}$$

$$\lambda^{-2} = k_1\{(\bar{C}_\alpha/D_\beta) + (\bar{C}_\beta/D_\alpha)\} + (k_2/D_\gamma) \qquad \text{(Eq. 8)}$$

In Eq. 8, $\bar{C}_\sigma$ refers to the stoichiometric concentration of species σ, when the reaction of Eq. 1 is at equilibrium and the J_R of Eq. 2 vanishes identically. The λ is defined as the relaxation length for the reaction in the system and is constant independent of position variable x due to the assumed constancy of diffusion coefficients. Equation 7 is a nonlinear differential equation for the reaction-rate profile in the inhomogeneous diffusional barrier under conditions of steady state. Blumenthal and Katchalsky (11) obtained the solution of Eq. 7, neglecting the nonlinear term $2k_1C_\alpha'C_\beta'$. A critical examination of this approximation is presented elsewhere (12).

Under conditions of steady states, one has from Eqs. 5*a–c* that:

$$C_\beta'' = (D_\alpha/D_\beta)C_\alpha'' \qquad \text{(Eq. 9}a\text{)}$$

$$C_\gamma'' = -(D_\alpha/D_\gamma)C_\alpha'' \qquad \text{(Eq. 9}b\text{)}$$

Under nonstationary-state conditions, one has:

$$C_\beta'' = (D_\alpha/D_\beta)C_\alpha'' + g_1 \qquad \text{(Eq. 10}a\text{)}$$

$$C_\gamma'' = -(D_\alpha/D_\gamma)C_\alpha'' + g_2 \qquad \text{(Eq. 10}b\text{)}$$

$$g_1 = (1/D_\beta)(\dot{C}_\alpha - \dot{C}_\beta) \qquad \text{(Eq. 10}c\text{)}$$

$$g_2 = -(1/D_\gamma)(\dot{C}_\alpha + \dot{C}_\gamma) \qquad \text{(Eq. 10}d\text{)}$$

The third basic assumption is now made—that both g_1 and g_2 are independent of the position variable. The implication of this assumption is that the departure of the state of the system from stationary states is small. If this assumption is valid, it is possible to express:

$$C_\beta(x) = (D_\alpha/D_\beta)C_\alpha(x) + (g_1/2)x^2 + r_1x + r_2 \qquad \text{(Eq. 11}a\text{)}$$

$$C_\gamma(x) = -(D_\alpha/D_\gamma)C_\alpha(x) + (g_2/2)x^2 + r_3x + r_4 \qquad \text{(Eq. 11}b\text{)}$$

Substitution of these equations into Eq. 2 enables one to express the reaction-rate profiles in a inhomogeneous diffusional barrier under conditions of nonstationary states as:

$$J_R(x) = \sum_{i=0} R_ix^i \qquad \text{(Eq. 12}a\text{)}$$

$$R_0 = J_R(0) = k_1C_\alpha(0)C_\beta(0) - k_2C_\gamma(0) \qquad \text{(Eq. 12}b\text{)}$$

$$R_1 = D_\alpha\eta^2a_1 + [k_1C_\alpha(0)p - k_2q] \qquad \text{(Eq. 12}c\text{)}$$

$$R_2 = D_\alpha\eta^2a_2 + k_1(D_\alpha/D_\beta)a_1^2 + k_1pa_1 + k_1C_\alpha(0)g_1/2 - k_2g_2/2 \qquad \text{(Eq. 12}d\text{)}$$

$$R_k = D_\alpha\eta^2a_k + k_1(D_\alpha/D_\beta)\sum_{\substack{i,j=1\\ i+j=k}}^{k-1} a_ia_j + k_1pa_{k-1} + (k_1g_1/2)a_{k-2} \qquad \text{(Eq. 12}e\text{)}$$

where:

$$r_1 = p = (1/D_\beta)(q_\beta - q_\alpha) \qquad \text{(Eq. 13}a\text{)}$$

$$r_2 = C_\beta(0) - (D_\alpha/D_\beta)C_\alpha(0) \qquad \text{(Eq. 13}b\text{)}$$

$$r_3 = q = (1/D_\gamma)(q_\alpha + q_\gamma) \qquad \text{(Eq. 13}c\text{)}$$

$$r_4 = C_\gamma(0) + (D_\alpha/D_\gamma)C_\alpha(0) \qquad \text{(Eq. 13}d\text{)}$$

$$\eta^2 = (k_2/D_\gamma) + (k_1/D_\alpha D_\beta)\{C_\alpha(0)D_\alpha + C_\beta(0)D_\beta\} \qquad \text{(Eq. 13}e\text{)}$$

$$a_i \equiv (1/i!)\{d^iC_\alpha(x)/dx^i\}|_{x=0} \qquad \text{(Eq. 13}f\text{)}$$

In these equations, $C_\alpha(0)$ is the concentration of species α at location $x = 0$, where inhomogeneity begins in the diffusional barrier. The constant parameter η^2 shares some similarity with λ^{-2} of Eq. 8 but is different from it; η becomes identical with λ^{-1} if the reaction of Eq. 1 is at equilibrium at location $x = 0$.

In a later section, the reaction-rate profile, $J_R^*(x)$, at steady state is evaluated as the solution of certain nonlinear differential equations and expressed as:

$$J_R^*(x) = \sum_{i=0} S_ix^i \qquad \text{(Eq. 14)}$$

Comparison of the expressions for constant coefficients S_i's and R_i's indicates that:

$$R_0 = S_0 \qquad R_1 = S_1 \qquad \text{(Eq. 15}a\text{)}$$

$$R_2 - S_2 = {}^1/_2\{k_1C_\alpha(0)g_1 - k_2g_2\} \qquad \text{(Eq. 15}b\text{)}$$

$$R_k - S_k = (k_1g_1/2)\,a_{k-2} \qquad k \geqslant 3 \qquad \text{(Eq. 15}c\text{)}$$

In other words, the departure of the state of the system from stationary states affects only coefficients of order greater than (x^2). So long as the reaction rate is either constant or a linear function of the position variable in the inhomogeneous diffusional barrier, the influence of chemical reactions on fluxes across a membrane approximates to the stationary-state calculations.

STATIONARY STATE (REACTION AT EQUILIBRIUM)

In this section, a nonlinear differential equation is derived for a function, $G(x)$, related to concentration profiles of the three species participating in the reaction; it is valid when diffusion coefficients are constants and coupling between fluxes may be ignored. On the basis of the assumption that the reaction of Eq. 1 is at equilibrium in the inhomogeneous diffusional barrier, the solution of the differential equation is analyzed, yielding relations between the observed flux of a specified species and the stability constant of the complex. The reaction can be at equilibrium in the inhomogeneous medium if and only if the concentration of one of the reacting species is maintained constant throughout the system. In other cases, the reaction rate of Eq. 2 cannot be nonvanishing inside the diffusional barrier under steady-state conditions subject to the validity of the assumptions contained in the derivation of the nonlinear differential equation for $G(x)$.

When one assumes that matter fluxes are independent of each other and proportional to their respective concentration gradients, as presented in Eq. 4, one has from Eq. 6:

$$-J_\alpha = I(x) + q_\alpha \quad \text{(Eq. 16a)}$$

$$-J_\beta = I(x) + q_\beta \quad \text{(Eq. 16b)}$$

$$J_\gamma = I(x) + q_\gamma \quad \text{(Eq. 16c)}$$

$$I(x) \equiv \int J_R^*(x)dx \quad \text{(Eq. 16d)}$$

$$J_\alpha^*(x) - J_\beta^*(x) = (q_\beta - q_\alpha) = \text{a constant} \quad \text{(Eq. 16e)}$$

$$J_\alpha^*(x) + J_\gamma^*(x) = (q_\gamma - q_\alpha) = \text{another constant} \quad \text{(Eq. 16f)}$$

If the species do not participate in the reaction to form a complex then the measured flux, J_σ, in the absence of a chemical reaction will equal $-q_\sigma$. When one has a mixture of chemically noninteracting species transporting across a diffusional barrier, the fluxes of each species are constant under stationary states. When three components of a mixture participate in the reaction of Eq. 1, then the fluxes of these three species are no longer independent of the position variable. However, linear combinations of the fluxes of two species, as presented in Eqs. 16*a–f*, remain constant independent of the position variable.

Equations 16*a–f*, along with Eq. 4 and the assumption of constant diffusion coefficients, yield the following relations for the concentration profiles of the three species:

$$C_\alpha(x) = (G/D_\alpha) + (q_\alpha/D_\alpha)x + K_\alpha \quad \text{(Eq. 17a)}$$

$$C_\beta(x) = (G/D_\beta) + (q_\beta/D_\beta)x + K_\beta \quad \text{(Eq. 17b)}$$

$$C_\gamma(x) = -(G/D_\gamma) - (q_\gamma/D_\gamma)x + K_\gamma \quad \text{(Eq. 17c)}$$

$$G \equiv G(x) = \int I(x)dx \quad \text{(Eq. 17d)}$$

where K_α, K_β, and K_γ are integration constants. It is evident that K_σ will equal $C_\sigma(0)$, if the constant term of any of the function $G(x)$ vanishes. Substituting expressions 17*a–d* into Eq. 2, one obtains the following nonlinear differential equation for $G(x)$:

$$G'' = \mu G^2 + \eta^2 G + \sigma G x + A x^2 + B x + J_R(0) \quad \text{(Eq. 18)}$$

where:

$$G'' = J_R^*(x)$$

$$J_R(0) = k_1 K_\alpha K_\beta - k_2 K_\gamma$$

$$\mu = (k_1/D_\alpha D_\beta)$$

$$\eta^2 = (k_2/D_\gamma) + \mu\{K_\alpha D_\alpha + K_\beta D_\beta\}$$

$$\sigma = \mu(q_\alpha + q_\beta)$$

$$A = \mu q_\alpha q_\beta$$

$$B = (k_2 q_\gamma/D_\gamma) + \mu\{K_\alpha D_\alpha q_\beta + K_\beta D_\beta q_\alpha\}$$

Equation 18 is valid for inhomogeneous regions provided the diffusion coefficients are constant and fluxes are given by Eq. 4. The coefficients μ, η^2, σ, A, B, and $J_R(0)$ are constants independent of the position variable x.

If the assumption that the reaction is at equilibrium inside every location of the diffusional barrier is valid, then it follows that:

$$G'' = J_R^*(x) = 0 \quad \text{(Eq. 19)}$$

Equation 19 demands that the function G, satisfying Eq. 18, can at best be a linear function of x, represented by:

$$G(x) = a + bx \quad \text{(Eq. 20)}$$

where a and b are constants. Substituting Eq. 20 into Eq. 18, one obtains

$$x^2 P + xQ + R = 0 \quad \text{(Eq. 21)}$$

$$P = \mu b^2 + \sigma b + A \quad \text{(Eq. 22a)}$$

$$Q = \eta^2 b + 2\mu a b + \sigma a + B \quad \text{(Eq. 22b)}$$

$$R = \mu a^2 + \eta^2 a + J_R(0) \quad \text{(Eq. 22c)}$$

Equation 21 is a polynomial in x, equal to zero. Recalling that in order for a polynomial of varying powers of x to equal zero, the coefficients of every power of x must identically vanish, one has $P = Q = R = 0$. One obtains from Eqs. 22*a* and 22*c* that:

$$a = -(\eta^2/2\mu) \pm (\eta^2/2\mu)[1 - \{4J_R(0)\mu/\eta^4\}]^{1/2} \quad \text{(Eq. 23a)}$$

$$b = -(\sigma/2\mu) \pm (\sigma/2\mu)[1 - \{4\mu A/\sigma^2\}]^{1/2} \quad \text{(Eq. 23b)}$$

Since the reaction is at equilibrium everywhere, one has

$$a = 0 \quad \text{or} \quad a = -(\eta^2/\mu) \quad \text{(Eq. 24a)}$$

$$b = -q_\alpha \quad \text{or} \quad b = -q_\beta \quad \text{(Eq. 24b)}$$

Substituting these results in Eq. 22*b*, which should also vanish, one obtains:

$$B = \eta^2 q \quad \text{when } b = -q_\alpha \text{ and } a = 0, \text{ or } -\eta^2/\mu \quad \text{(Eq. 25a)}$$

$$B = \eta^2 q_\alpha \quad \text{when } b = -q_\beta \text{ and } a = 0, \text{ or } -\eta^2/\mu \quad \text{(Eq. 25b)}$$

The two expressions for B and $\eta^2 q_\alpha$ obtained from Eq. 18 are equal if either:

$$q_\alpha = q_\beta = q_\gamma \quad \text{(Eq. 26)}$$

or:

$$q_\alpha = \{k_1 D_\gamma q_\beta + k_2 D_\beta q_\gamma\}\{k_2 D_\beta + k_1 K_\alpha D_\gamma\}^{-1} \quad \text{(Eq. 27)}$$

Similarly, the two expressions B and $\eta^2 q_\beta$ become identical if either Eq. 26 is satisfied or:

$$q_\beta = \{k_2 D_\alpha q_\gamma + k_1 K_\beta D_\gamma q_\alpha\}\{k_2 D_\alpha + k_1 K_\beta D_\gamma\}^{-1} \quad \text{(Eq. 28)}$$

The validity of Eq. 26 along with Eqs. 17*a–d* and 4 demands that the gradients of all three species vanish across the diffusional barrier. In other words, Eq. 26 describes the equilibrium state of the whole system. When one chooses the value $b = -q_\beta$, one has:

$$C_\beta' = 0 \quad \text{(Eq. 29a)}$$

$$C_\alpha' = (q_\alpha - q_\beta)/D_\alpha \quad \text{(Eq. 29b)}$$

The rigorous validity of Eq. 24*b*, when the reaction is at equilibrium in the diffusional barrier, and constant diffusion coefficients require that the concentration gradient of either α or β should vanish. Conversely, if the gradients of the reacting species α and β do not vanish across the diffusional barrier, then Eq. 20 cannot be a solution of Eq. 18 and, therefore, the reaction cannot be at equilibrium in the inhomogeneous diffusional barrier. In addition, the reaction rate cannot even be constant in the inhomogeneous diffusional barrier if either of the gradients of the reacting species does not vanish. Inspection of Eq. 18 suggests that under no circumstance can the function G be a quadratic function of x.

Assuming that experiments are carried out maintaining the concentration of β the same on either side of the membrane, and that measurements of flux of α, J_α^*, are obtained in the presence of vary-

ing concentrations of β on both sides of the membrane, one has from Eq. 28:

$$J_\alpha^*(C_\beta) = (q_\gamma - q_\alpha)\{1 + KK_\beta(D_\gamma/D_\alpha)\}^{-1} \quad \text{(Eq. 30)}$$

where $K = (k_1/k_2)$ and $K_\beta = C_\beta{}^l$ when $a = 0$.

If $C_\beta = 0$, then the flux of α would have been:

$$J_\alpha{}^{0*} = J_\alpha^*(C_\beta = 0) = -q_\alpha \quad \text{(Eq. 31)}$$

Thus, in the presence of β, the additional flux of α caused by the complex-forming reaction in the system, when the reaction is at equilibrium everywhere, is given by:

$$J_\alpha^*(C_\beta) - J_\alpha^*(C_\beta = 0) = \{KC_\beta D_\gamma q_\alpha - q_\gamma D_\alpha\} \times [D_\alpha + KC_\beta D_\gamma]^{-1} \quad \text{(Eq. 32)}$$

Therefore, from the measurements of fluxes J_α, under steady state in the presence of known concentrations of β on either side of the diffusional barrier, and knowledge of D_α, D_γ, q_α, and q_γ, one can compute the stability constant K of the complex using Eq. 22; q_α and q_γ are the negatives of the fluxes of species α and γ obtained for the same diffusional barrier in the absence of the other two species.

The conclusions of this section and Eq. 22 may be independently derived by the following simpler argument. Under stationary state, when the reaction rate vanishes, one has from Eqs. 4 and 6 that $C_\gamma{}'$ should be a constant when D_γ is a constant. However, from Eq. 2, one has:

$$C_\gamma{}' = (k_1/k_2)\{C_\alpha C_\beta{}' + C_\beta C_\alpha{}'\} \quad \text{(Eq. 33)}$$

The right-hand side of Eq. 33 will be a constant if and only if either $C_\alpha{}'$ or $C_\beta{}'$ vanishes. When $C_\beta{}' = 0$, one has from Eqs. 9*a–b* and 33:

$$P = (q_\alpha + q_\gamma)/D_\gamma = \{(KC_\beta D_\gamma + D_\alpha)/D_\gamma\}C_\alpha{}' \quad \text{(Eq. 34)}$$

Hence,

$$J_\alpha(C_\beta) - J_\alpha(C_\beta = 0) = q_\alpha - [PD_\gamma D_\alpha/(KC_\beta D_\gamma + D_\alpha)] \quad \text{(Eq. 35)}$$

Equation 35 leads to Eq. 32.

STATIONARY-STATE REACTION-RATE PROFILE

By assuming that the concentration of species α can be expanded in a Taylor series in terms of its concentration and derivatives about the position $x = 0$, the dependence of the reaction-rate profile in the inhomogeneous diffusional barrier on the position variable under nonsteady-state conditions was presented in Eqs. 12*a–e*. In this section, corresponding expressions will be obtained for steady states, valid when diffusion coefficients are constants and coupling between fluxes of different species is ignored. Instead of solving the nonlinear differential Eq. 7, the procedure involves finding solution of Eq. 18. Thus, both the concentration profiles and the reaction-rate profiles in the inhomogeneous region are obtained. As shown here, concentration and reaction-rate profiles can be computed to any order of powers of the position variable that one may desire, subject to an approximation presented in this section. Since the solutions on either side of the diffusional barrier are well stirred, the solutions on either side of the barrier can be regarded as homogeneous; concentrations of the species remain uniform up to a region close to what one may call the boundaries of the membrane. It is the inhomogeneous character of the diffusional barrier that is responsible for the maintenance of concentration profiles and reaction-rate profiles under steady state.

Let the plane of the barrier where inhomogeneity begins correspond to the value of the position variable $x = 0$. The boundaries of inhomogeneity extend from $x = 0$ to $x = h$, where h is the thickness of the diffusional barrier. Since the concentrations of the species are analytical functions of the position variable, one may assume that the concentrations of the three species participating in the reaction at location x may be represented in terms of the concentrations of the species at $x = 0$ as:

$$C_\alpha(x) = C_\alpha(0) + a_1x + \sum_{i=2} a_ix^i \quad \text{(Eq. 36a)}$$

$$C_\beta(x) = C_\beta(0) + b_1x + \sum_{i=2} b_ix^i \quad \text{(Eq. 36b)}$$

$$C_\gamma(x) = C_\gamma(0) + c_1x + \sum_{i=2} c_ix^i \quad \text{(Eq. 36c)}$$

$$b_i = (1/i!)[d^iC_\beta/dx^i]|_{x=0} \quad \text{(Eq. 36d)}$$

Substituting Eqs. 36*a–d* into Eq. 2, one obtains:

$$J_R^*(x) = \sum_{i=0} S_ix^i \quad \text{(Eq. 37a)}$$

$$S_0 = J_R^*(0) = k_1C_\alpha(0)C_\beta(0) - k_2C_\gamma(0) \quad \text{(Eq. 37b)}$$

$$S_1 = k_1\{C_\beta(0)a_1 + C_\alpha(0)b_1\} - k_2c_1 \quad \text{(Eq. 37c)}$$

$$S_2 = k_1\{C_\beta(0)a_2 + C_\alpha(0)b_2 + a_1b_1\} - k_2c_2 \quad \text{(Eq. 37d)}$$

$$S_3 = k_1\{C_\beta(0)a_3 + C_\alpha(0)b_3 + a_1b_2 + a_2b_1\} - k_2c_3 \quad \text{(Eq. 37e)}$$

. . .
. . .

Validity of Eqs. 9*a–b* under steady states and the assumptions about constant diffusion coefficients requires that the Taylor expansion coefficients a_i, b_i, and c_i are related by the relations:

$$b_i = (D_\alpha D_\beta)a_i \qquad b_1 = (D_\alpha/D_\beta)a_1 + p \quad \text{(Eq. 38a)}$$

$$c_i = -(D_\alpha/D_\gamma)a_i \qquad c_1 = -(D_\alpha/D_\gamma)a_1 + q \quad \text{(Eq. 38b)}$$

$$p = (1/D_\beta)\{J_\alpha(x) - J_\beta(x)\} \quad \text{(Eq. 38c)}$$

$$q = -(1/D_\gamma)\{J_\alpha(x) + J_\gamma(x)\} \quad \text{(Eq. 38d)}$$

Both p and q are constants.

Let the solution satisfying the nonlinear differential Eq. 18 be:

$$G(x) = \sum_{i=0} m_ix^i \quad \text{(Eq. 39)}$$

If Eq. 39 satisfies Eq. 18 identically, and if one has means of evaluating the coefficients of every power of x of Eq. 39, one has solved the problem. Substitution of Eq. 39 in Eq. 18 yields the relations:

$$\mu\left\{\sum_{i=0}\sum_{j=0} m_im_jx^{i+j}\right\} + \eta^2\sum_{i=0} m_ix^i + \sigma\sum_{i=0} m_ix^{i+1} + Ax^2 + Bx + J_R^*(0) - \sum_{i=2} i(i-1)m_ix^{i-2} = 0 \quad \text{(Eq. 40a)}$$

$$J_R^*(x) = G'' = \sum_{i=2} i(i-1)m_ix^{i-2} = \sum_{i=0} S_ix^i \quad \text{(Eq. 40b)}$$

Equating the coefficients of like powers of x, one obtains:

$$\mu m_0{}^2 + \eta^2m_0 = 2m_2 - J_R^*(0) \quad \text{(Eq. 41a)}$$

$$2\mu m_0m_1 + \eta^2m_1 + \sigma m_0 + B - S_1 = 0 \quad \text{(Eq. 41b)}$$

$$\mu(m_1{}^2 + 2m_0m_2) + \eta^2m_2 + \sigma m_1 + A - S_2 = 0 \quad \text{(Eq. 41c)}$$

$$2\mu(m_0m_3 + m_1m_2) + \eta^2m_3 + \sigma m_2 - S_3 = 0 \quad \text{(Eq. 41d)}$$

$$\mu\sum_{\substack{i,j=0\\ i+j=k}}^{k} m_im_j + \eta^2m_k + \sigma m_{k-1} - S_k = 0 \quad k \geqslant 3 \quad \text{(Eq. 41e)}$$

Since, $\lim_{x\to 0} J_R^*(x) = J_R^*(0) = 2m_2$, one has from Eq. 41*a* that:

$$m_0 = -(\eta^2/\mu) \quad \text{(Eq. 42)}$$

or

$$m_0 = 0 \quad \text{(Eq. 43)}$$

One has from Eq. 41*c* that:

$$m_1 = -(\sigma/2\mu) \pm (\sigma/2\mu)[1 - (4\mu A/\sigma^2) + (4\mu/\sigma^2)\{S_2 - \eta^2m_2 - 2\mu m_0m_2\}]^{1/2}$$
$$= -(q_\alpha + q_\beta)/2 \pm {}^1/_2[(q_\alpha - q_\beta)^2 + (4/\mu)(S_2 - \eta^2m_2) - 8m_0m_2]^{1/2} \quad \text{(Eq. 44)}$$

Recall that (*cf.*, Eqs. 48*a–d*):

$$S_2 - \eta^2m_2 = S_2 - D_\alpha\eta^2a_2 = k_1a_1b_1 \quad \text{(Eq. 45)}$$

Hence, when $m_0 = 0$ or $m_2 = 0$,

$$m_1 = -(q_\alpha + q_\beta)/2 \pm {}^1/_2[(q_\alpha - q_\beta)^2 + 4D_\alpha D_\beta a_1 b_1]^{1/2} \quad \text{(Eq. 46)}$$

Upon rearrangement and squaring both sides, one obtains:

$$D_\alpha D_\beta a_1 b_1 = m_1^2 + m_1(q_\alpha + q_\beta) + q_\alpha q_\beta \quad \text{(Eq. 47a)}$$

$$m_2 \text{ or } m_0 = 0 \quad \text{(Eq. 47b)}$$

From Eqs. 38*a–d*, the constant coefficients S_i's of Eqs. 37*a–e* may be reexpressed as:

$$S_0 = k_1 K_\alpha K_\beta - k_2 K_\gamma \quad \text{(Eq. 48a)}$$

$$S_1 = D_\alpha \eta^2 a_1 + k_1 C_\alpha(0) p - k_2 q \quad \text{(Eq. 48b)}$$

$$S_2 = D_\alpha \eta^2 a_2 + k_1 a_1 b_1 \quad \text{(Eq. 48c)}$$

$$S_k = D_\alpha \eta^2 a_k + k_1(D_\alpha/D_\beta) \sum_{\substack{i,j=1 \\ i+j=k}}^{k-1} a_i a_j + k_1 p a_{k-1} \quad \text{(Eq. 48d)}$$

where $k \geq 3$, $p = (q_\beta - q_\alpha)/D_\beta$, and $q = (q_\alpha + q_\gamma)/D_\gamma$. In the limit x tending to zero, one has from Eqs. 17*a–d*, when $m_0 = -(\eta^2/\mu)$,

$$C_\alpha(0) = K_\alpha - (\eta^2 D_\beta/k_1) \quad \text{(Eq. 49a)}$$

$$C_\beta(0) = K_\beta - (\eta^2 D_\alpha/k_1) \quad \text{(Eq. 49b)}$$

$$C_\gamma(0) = K_\gamma + (\eta^2/\mu D_\gamma) \quad \text{(Eq. 49c)}$$

$$K_\sigma = C_\sigma^I \qquad \sigma = \alpha,\beta,\gamma \quad \text{(Eq. 49d)}$$

When $m_0 = 0$, one has

$$K_\sigma = C_\sigma(0) = C_\sigma^I \qquad \sigma = \alpha,\beta,\gamma \quad \text{(Eq. 50)}$$

where C_σ^I is the concentration of species σ in the bulk homogeneous region on the left-hand side of the diffusional barrier. One may verify that S_0, the reaction rate at location zero, $J_R^*(0)$, is given by Eq. 48*a*, irrespective of whether Eq. 49 or 50 is utilized in the expression 37*b*.

Utilizing the expressions for the parameters presented in Eqs. 18, 13, and 12 into Eq. 48*b*, one evaluates:

$$B - S_1 = \eta^2 D_\alpha a_1 + \eta^2 q_\beta + (2k_2 q_\gamma/D_\gamma)$$
$$= \eta^2 D_\beta b_1 + \eta^2 q_\alpha + (2k_2 q_\gamma/D_\gamma) \quad \text{(Eq. 51a)}$$

when $m_0 = -(\eta^2/\mu)$, and

$$S_1 - B = \eta^2 D_\alpha a_1 - \eta^2 q_\alpha - (2k_2 q_\gamma/D_\gamma)$$
$$= \eta^2 D_\beta b_1 - \eta^2 q_\beta - (2k_2 q_\gamma/D_\gamma) \quad \text{(Eq. 51b)}$$

when $m_0 = 0$.

From Eqs. 51*a–b* and 41*b*, one obtains:

$$m_1 = D_\alpha a_1 + (2k_2 q_\gamma/D_\gamma \eta^2) - q_\alpha \quad \text{(Eq. 52a)}^1$$
$$= D_\beta b_1 + (2k_2 q_\gamma/D_\gamma \eta^2) - q_\beta \quad \text{(Eq. 52b)}$$

when $m_0 = -(\eta^2/\mu)$, and

$$m_1 = D_\alpha a_1 - q_\alpha + (2k_2 q_\gamma/D_\gamma \eta^2) \quad \text{(Eq. 52c)}$$
$$= D_\beta b_1 - q_\beta + (2k_2 q_\gamma/D_\gamma \eta^2) \quad \text{(Eq. 52d)}$$

when $m_0 = 0$. Equations 52*a–d* yield the result: $(D_\alpha a_1 - D_\beta b_1 = q_\alpha - q_\beta)$, in agreement with Eqs. 12*a–e* and 38*a–d*.

Equations 52*a–d* yield m_1 in terms of a_1 or b_1. It is convenient to express m_1 in terms of experimentally available quantities. This is achieved by utilizing the following relation, easily derived from Eq. 46, valid when $m_0 = 0$:

$$D_\alpha D_\beta a_1 b_1 = (m_1 + q_\alpha)(m_1 + q_\beta) \quad \text{(Eq. 53)}$$

Substituting Eqs. 52*c* and 52*d* into Eq. 53, one obtains:

$$D_\alpha a_1 + D_\beta b_1 = (2k_2 q_\gamma/D_\gamma \eta^2) \qquad m_0 = 0 \quad \text{(Eq. 54)}$$

Thus, when $m_0 = 0$, one evaluates:

$$D_\alpha a_1 = (q_\alpha - q_\beta)/2 + (k_2 q_\gamma/D_\gamma \eta^2) \quad \text{(Eq. 55a)}$$

$$D_\beta b_1 = (q_\beta - q_\alpha)/2 + (k_2 q_\gamma/D_\gamma \eta^2) \quad \text{(Eq. 55b)}$$

$$m_1 = -(q_\alpha + q_\beta)/2 + (k_2 q_\gamma/D_\gamma \eta^2) \quad \text{(Eq. 55c)}$$

When $m_0 = -(\eta^2/\mu)$, one obtains from Eq. 41*c*:

$$(m_1 + q_\alpha)(m_1 + q_\beta) = D_\alpha D_\beta a_1 b_1 + (2\eta^2 m_2/\mu) \quad \text{(Eq. 56)}$$

Utilizing Eqs. 52*a* and 52*b* in Eq. 56, one evaluates:

$$D_\alpha a_1 + D_\beta b_1 = (\eta^4 D\gamma m_2/k_2 \mu q_\gamma) - (2k_2 q_\gamma/D_\gamma \eta^2) \quad \text{(Eq. 57a)}$$

$$D_\alpha a_1 - D_\beta b_1 = q_\alpha - q_\beta \quad \text{(Eq. 57b)}$$

Therefore, when $m_0 = -(\eta^2/\mu) \neq 0$, one has:

$$D_\alpha a_1 = (q_\alpha - q_\beta)/2 - (k_2 q_\gamma/D_\gamma \eta^2) + \zeta \quad \text{(Eq. 58a)}$$

$$D_\beta b_1 = (q_\beta - q_\alpha/2 - (k_2 q_\gamma/D_\gamma \eta^2) + \zeta \quad \text{(Eq. 58b)}$$

$$m_1 = -(q_\alpha + q_\beta)/2 + (k_2 q_\gamma/D_\gamma \eta^2) + \zeta \quad \text{(Eq. 58c)}$$

where $\zeta = \eta^4 D_\gamma m_2/(2\, k_2 \mu q_\gamma)$.

For Eqs. 51–58, the parameters B and η^2 are given by Eq. 18, S_1 is given by Eq. 37*c* and $K_\sigma = C_\sigma(0)$, when $m_0 = 0$, and $K_\alpha = C_\alpha(0) + (\eta^2 D_\beta/k_1)$, $K_\beta = C_\beta(0) + (\eta^2 D_\alpha/k_1)$ when $m \neq 0$.

From Eq. 40*b*, one has, by equating coefficients of like powers of position variables, the relation:

$$S_k = (k + 2)(k + 1) m_{k+2} \qquad k \geq 0 \quad \text{(Eq. 59)}$$

From the nonlinear differential equation:

$$D_\alpha C_\alpha'' = J_R^*(x) \quad \text{(Eq. 60)}$$

one has:

$$S_k = (k + 2)(k + 1) D_\alpha a_{k+2} \quad \text{(Eq. 61)}$$

Thus,

$$D_\alpha a_k = m_k \qquad k \geq 2 \quad \text{(Eq. 62)}$$

Equations 41*a–e* can be reexpressed for $k \geq 3$ as:

$$S_k - m_k(\eta^2 + 2\mu m_0) = \mu \sum_{\substack{i,j=1 \\ i+j=k}}^{k-1} m_i m_j + \sigma m_{k-1} \quad \text{(Eq. 63)}$$

Thus, knowledge of $m_0, m_1, m_2 \ldots m_{k-1}$ completely determines the right-hand side of Eq. 63. The left-hand side of Eq. 63 equals $(S_k \pm \eta^2 m_k)$, the positive sign being applicable when $m_0 = -(\eta^2/\mu)$ and the negative sign being valid when $m_0 = 0$. From Eqs. 59 and 63, one has:

$$S_k = \mp\{\eta^2/k(k-1)\} S_{k-2} + \mu \sum_{\substack{i,j=1 \\ i+j=k}}^{k-1} m_i m_j + \sigma m_{k-1} \quad \text{(Eq. 64)}$$

Equation 64 expresses the fact that knowledge of lower order coefficients, S_i's, enables one to compute higher order coefficients. For example, when $k = 4$, one has:

$$S_4 = \mp(\eta^2/12) S_2 + (\mu/4) S_0^2 + (S_1/6)\mu(q_\alpha + q_\beta + 2m_1) \quad \text{(Eq. 65a)}$$

$$\mu = (k_1/D_\alpha D_\beta) \quad \text{(Eq. 65b)}$$

In Eq. 65*a*, the negative sign is valid when $m_0 = -(\eta^2/\mu)$ and the positive sign is valid when $m_0 = 0$.

To summarize the results obtained so far, the interest is in computing the reaction-rate profiles in the inhomogeneous diffusional barrier as a function of the position variable, presented in Eqs. 37*a–e*, satisfying the differential Eq. 18. Equations 37*a–e* are of little use unless one can compute the coefficients in terms of experimen-

[1] *Note added in proof:* The value of m_1 presented in Eq. 52 is only an *approximation* and is not a unique solution. m_1 equals $-q_\alpha$, $-q_\beta$, or $\pm q_\gamma$, respectively, when a_1, b_1, or c_1 vanishes. m_1 assumes null value when diffusion coefficients vary with position. When diffusion coefficients are constants and a_1, b_1, and c_1 are nonvanishing, determination of a unique value for m_1 has not yet been achieved.

tally measurable quantities. Equation 64 enables one to compute any desired coefficient $S_k (k \geq 3)$, in terms of already obtained lower-order coefficients.

When $m_0 = 0$, one has obtained:

$$S_0 = k_1 K_\alpha K_\beta - k_2 K_\gamma \qquad K_\sigma = C_\sigma^I \qquad \text{(Eq. 66a)}$$

$$S_1 = m_1 \eta^2 + B \qquad \text{(Eq. 66b)}$$

$$S_2 = \mu\{m_1^2 + m_1(q_\alpha + q_\beta) + q_\alpha q_\beta\} + (\eta^2 S_0/2) \qquad \text{(Eq. 66c)}$$

$$S_3 = \eta^2 m_3 + (S_0\mu/2)\{q_\alpha + q_\beta + 2m_1\} \qquad \text{(Eq. 66d)}$$

$$S_k = \eta^2 m_k + \mu\{q_\alpha + q_\beta + 2m_1\} m_{k-1} + \mu \sum_{\substack{i,j=2 \\ i+j=k}}^{k-2} m_i m_j \qquad k \geq 3 \qquad \text{(Eq. 66e)}$$

The knowledge of the rate constants (k_1 and k_2), diffusion coefficients (D_α, D_β, and D_γ) of the three species participating in the reaction measured in homogeneous bulk phase, concentrations of these species (K_σ) in the left-hand side of the membrane, and the fluxes of the species (q_σ) measured in the absence of the other two species across the same diffusional barrier enables one to compute S_0, S_1, and S_2 using Eqs. 66*a–e*, valid when $m_0 = 0$. The value of m_1 required for the said computation is presented in Eq. 55*c*.

When $m_0 = -(\eta^2/\mu)$, one has:

$$S_0 = k_1 K_\alpha K_\beta - k_2 K_\gamma \qquad \text{(Eq. 67a)}$$

$$S_1 = -m_1 \eta^2 + B - \eta^2(q_\alpha + q_\beta) \qquad \text{(Eq. 67b)}$$

$$S_2 = \mu\{m_1^2 + m_1(q_\alpha + q_\beta) + q_\alpha q_\beta\} - (\eta^2 S_0/2) \qquad \text{(Eq. 67c)}$$

$$S_3 = -m_3 \eta^2 + (S_0\mu/2)\{q_\alpha + q_\beta + 2m_1\} \qquad \text{(Eq. 67d)}$$

$$S_k = -m_k \eta^2 + \mu(q_\alpha + q_\beta + 2m_1) m_{k-1} + \mu \sum_{\substack{i,j=2 \\ i+j=k}}^{k-2} m_i m_j \qquad k \geq 3 \qquad \text{(Eq. 67e)}$$

In Eqs. 67*a–e*, $K_\sigma = C_\sigma^I \neq C_\sigma(0)$. Thus, $S_0 = 2m_2$ can be computed from knowledge of rate constants k_1 and k_2 and concentrations of the species in the left-hand side of the membrane; m_1 should be computed using Eq. 44. The parameter η^2 required for the calculation is obtained from Eq. 18. Knowledge of S_0, S_1, and S_2, obtained using Eqs. 67*a–e*, can be utilized to obtain any S_k as indicated in Eqs. 64 and 65*a–b*. However, one needs knowledge of S_2 to solve for m_1 using Eq. 44, and knowledge of m_1 is required to solve for S_2 as indicated in Eq. 67*c*. Thus, in the general case, when $m_0 \neq 0$ and $m_2 \neq 0$, one must resort to an iterative procedure suggested elsewhere (12, 13). It is convenient to make the approximation that when $m_0 \neq 0$, $m_2 = 0$ and compute m_1 using Eq. 58*c*.

DISCUSSION

The analysis presented in this paper is based on the assumption that the three species transporting across a diffusional barrier can participate in a reaction of the type presented in Eq. 1. In the second section, it was shown that the reaction-rate profile in the inhomogeneous region can be expressed as a polynomial in the position variable, presented in Eqs. 12*a–e*, satisfying the dictation of equations of continuity when the three basic assumptions mentioned in the section are valid. It was shown that if the departure of the state of the system from stationary state can be regarded as small, it suffices to know the reaction-rate profile in the inhomogeneous diffusional barrier under conditions of stationary state.

In the third section, a nonlinear differential equation (Eq. 18) for a function $G(x)$, related to concentration profiles and reaction-rate profiles in the inhomogeneous region, was derived. Equation 18 is valid for stationary states when diffusion coefficients are constants and coupling between fluxes of different species can be ignored. On the basis of the solution of Eq. 18 and the usual assumption incorporated that the reaction is at equilibrium at all locations in the system, it was shown that the reaction cannot be at equilibrium in the inhomogeneous region except when the concentration gradient of one of the reactant species vanishes across the diffusional barrier.

When experiments are conducted that maintain the concentration of one of the reactant species equal on both sides of the membrane, the relation between the flux of the other reactant species and the stability constant of the complex was presented in Eq. 32.

In the fourth section, the general solution of Eq. 18, when the reaction rate is nonvanishing in inhomogeneous regions, under conditions of stationary state, was obtained. In that section, it was indicated how the reaction-rate profile can be computed to any order of accuracy one desires by using experimentally available information. Thus, when the concentrations of the species at the location where inhomogeneity sets in equal the bulk concentrations [$C_\sigma(0) = C_\sigma^I$; $m_0 = 0$], or when the reaction is at equilibrium at the location where inhomogeneity begins ($m_2 = 0$), one can safely assert that the coefficients S_i's of Eqs. 37*a–e* can be computed and thus are known.

The fluxes of the three species across the diffusional barrier under stationary-state conditions are given by:

$$J_\alpha = -q_\alpha - I(x) \qquad \text{(Eq. 68a)}$$

$$J_\beta = -q_\beta - I(x) \qquad \text{(Eq. 68b)}$$

$$J_\gamma = q_\gamma + I(x) \qquad \text{(Eq. 68c)}$$

$$I(x) = \sum_{i=0} \{S_i/(i+1)\} x^{i+1} \qquad \text{(Eq. 68d)}$$

If the diffusional barrier extends from $x = 0$ to $x = h$, and the flux is measured in the right-hand side of the membrane, the observed fluxes will be:

$$J_\alpha(h) = -q_\alpha - I(h) \qquad \text{(Eq. 69a)}$$

$$J_\beta(h) = -q_\beta - I(h) \qquad \text{(Eq. 69b)}$$

$$J_\gamma(h) = q_\gamma + I(h) \qquad \text{(Eq. 69c)}$$

$$I(h) = I(x = h) \qquad \text{(Eq. 69d)}$$

where $I(h)$ indicates the production or consumption of species in the inhomogeneous region due to the reaction, and h is of the order of about 100 Å for lipid films and many biological membranes.

The experiments of Reuning and Levy (1–3) yielded information about the rate of accumulation of, for example, species α in the second compartment, the nylon bag. Since the flux, J_α^*, indicates the number of moles of α transferred per unit area per unit time, the product J_α^* multiplied by area of the diffusional barrier should equal the rate of accumulation of the species in the nylon bag. Assuming that the area of the diffusional barrier remains constant, measured (dC_α^{II}/dt) is proportional to J_α^*, under conditions of steady state:

$$(dC_\alpha^{II}/dt) = J_\alpha(h)A \qquad \text{(Eq. 70)}$$

If the experiments are conducted with varying amounts of C_β^I in the left-hand side of the diffusional barrier and one measures (dC_α^{II}/dt) for the same diffusional barrier, one can compute $(d/dC_\beta^I)(dC_\alpha^{II}/dt)$. This quantity should equal the right-hand side of Eq. 71 according to the theory presented in this paper, subject to neglect of (h^4) order and higher order terms. The right-hand side of Eq. 71 can be computed from independently obtainable experimental quantities:

$$\frac{d}{dC_\beta^I}\left(\frac{dC_\alpha^{II}}{dt}\right) = -Ah[k_1 C_\alpha^I + h\{(q_\beta - q_\alpha)/2 - (k_2 q_\gamma/D_\gamma \eta^2)\} + (k_1/2)\{\eta^2 h^2 C_\alpha^I + (S_0 h^2/D_\alpha)\}] + 0(h^4) \qquad \text{(Eq. 71)}$$

In obtaining the right-hand side of Eq. 71, one utilizes the relations:

$$m_0 = 0;\ C_\sigma^I = K_\sigma;\ \sigma = \alpha,\beta,\gamma \qquad \text{(Eq. 72a)}$$

$$(dS_0/dK_\beta) = k_1 C_\alpha^I \qquad \text{(Eq. 72b)}$$

$$(dS_1/dK_\beta = (q_\beta - q_\alpha)/2 - (k_2 q_\gamma/D_\gamma \eta^2) \qquad \text{(Eq. 72c)}$$

$$(dS_2/dK_\beta) = (k_1/2)\{\eta^2 K_\alpha + (S_0/D_\alpha)\} \qquad \text{(Eq. 72d)}$$

$$S_0 = k_1 K_\alpha K_\beta - k_2 K_\gamma \qquad \text{(Eq. 72e)}$$

$$S_1 = m_1 \eta^2 + B \qquad \text{(Eq. 72f)}$$

$$S_2 = \mu\{m_1^2 + m_1(q_\alpha + q_\beta) + q_\alpha q_\beta\} + (\eta^2 S_0/2) \qquad \text{(Eq. 72g)}$$

If one now adopts the approximation that (h^3) order and higher order terms may be neglected, it is evident from Eq. 71 that the slope of the plot of the quantity $(Ah)^{-1}\left\{\frac{d}{dC_\beta^I}\left(\frac{dC_\alpha^{II}}{dt}\right)\right\}$ against C_α^I

will equal the negative of the association-rate constant k_1. The intercept of the said plot will enable one to compute the dissociation-rate constant k_2 from knowledge of $q_\alpha, q_\beta, q_\gamma$, and D_γ.

REFERENCES

(1) R. H. Reuning and G. Levy, *J. Pharm. Sci.*, **57**, 1342(1968).
(2) *Ibid.*, **57**, 1556(1968).
(3) *Ibid.*, **58**, 79(1969).
(4) D. Fitts, "Nonequilibrium Thermodynamics," McGraw-Hill, New York, N. Y., 1962.
(5) V. S. Vaidhyanathan, *J. Theoret. Biol.*, **8**, 344(1965).
(6) *Ibid.*, **9**, 478, 489(1965).
(7) L. Onsager and R. M. Fuoss, *J. Phys. Chem.*, **36**, 2689 (1932).
(8) V. S. Vaidhyanathan, in "Intracellular Transport," H. B. Warren, Ed., Academic, New York, N. Y., 1966.
(9) V. S. Vaidhyanathan, *J. Theoret. Biol.*, **23**, 232(1969).
(10) R. J. Bearman, *J. Chem. Phys.*, **29**, 1278(1958).
(11) R. Blumenthal and A. Katchalsky, *Biochim. Biophys. Acta*, **173**, 357(1969).
(12) V. S. Vaidhyanathan, to be published.
(13) V. S. Vaidhyanathan and M. Seshadri, *Biochim. Biophys. Acta*, **211**, 1(1970).

ACKNOWLEDGMENTS AND ADDRESSES

Received December 2, 1970, from the *Departments of Pharmaceutics and Biophysics, School of Pharmacy and Center for Theoretical Biology, State University of New York at Buffalo, Amherst, NY 14226*

Accepted for publication February 23, 1971.

Supported by a grant from the Life Insurance Medical Research Fund.

Presented to the Basic Pharmaceutics Section, APhA Academy of Pharmaceutical Sciences, Washington, D.C. meeting, November 1970.

Pharmacodynamics of Chemotherapeutic Effects: Dose–Time–Response Relationships for Phase-Nonspecific Agents

WILLIAM J. JUSKO

Abstract □ Pharmacodynamic relationships were developed to characterize the necrobiotic effects of phase-nonspecific chemotherapeutic agents which attach irreversibly to cell receptors. The site of drug action is considered to be a specific body compartment, and target cell inactivation by the agent results from a bimolecular drug–receptor interaction. Turnover of cells is assumed to occur by natural synthetic and degradative processes. Based on these premises, a log–linear relationship was evolved to relate the fraction of surviving cells to the drug level–time integral at the pharmacologic site. The integral was shown to be proportional to the dose and independent of the mode of administration when the entire drug level–time course is evaluated. Data from the literature for the effects of cyclophosphamide on three cell systems of mice demonstrate the usefulness and certain therapeutic implications of the equations.

Keyphrases □ Pharmacodynamics—dose–time–response relationships, phase-nonspecific agents □ Chemotherapeutic agents, phase nonspecific—pharmacodynamic model □ Dose–effect relationships—cyclophosphamide on cell systems of mice

Considerable progress has been made in the development of kinetic relationships characterizing pharmacologic effects. Levy (1) showed that the intensity *versus* time course of many clinically observable pharmacologic effects may be described adequately by mathematical expressions based on the kinetics of drug elimination and on the established relationship between amount in the body and response. In turn, the simultaneous use of pharmacologic effect and pharmacokinetic data was shown to be an added dimension in the analysis of pharmacodynamic data (2).

The pharmacologic response to most drugs can be quantitated in a log dose–linear effect manner. Such a relationship is essentially derived from the postulation of reversible interaction between drug and receptor (3, 4). The reversibility aspect of this mechanism precludes application of most classical pharmacodynamic principles to therapy with certain antibiotics, antimetabolites, and alkylating agents. The cytotoxic effects of such agents are usually dependent on the irreversible or covalent incorporation of drug into cell metabolic sites or pathways (5). The lack of a mathematical basis for predicting the clinical effects of chemotherapy and the clinical difficulties in measuring such effects have been partly responsible for the uncertainty involved in the design of appropriate dosage modes and schedules for chemotherapeutic agents (4). The purpose of this report is to develop pharmacodynamic principles that may be of quantitative and predictive value in the therapeutic use of such drugs.

THEORETICAL

A basic pharmacodynamic model for the characterization of the effects of chemotherapeutic agents is shown in Scheme I. The drug is introduced into the central compartment (X_c) using a suitable mode of administration. The site of chemotherapeutic effect (X_t) is considered to be a homogeneous compartment separate from the central volume of distribution. First-order transfer-rate constants between the two compartments are k_{12} and k_{21}, and the elimination-rate constant is k_{el}. A portion of the dose of drug that reaches the pharmacologic site is involved in an irreversible reaction (rate con-